

Hybridization and Introgression in Tiger Salamanders



*by Robb F. Leary
Montana Fish, Wildlife & Parks
Division of Biological Services
University of Montana
Missoula, Montana 59812*

Table of Contents

[Hybridization and Introgression in Tiger Salamanders](#)

[Introduction](#)

[Tiger Salamanders](#)

[California Tiger Salamanders](#)

[The Research](#)

[The Listing](#)

[Barred Tiger Salamanders](#)

[Investigating Hybridization](#)

[Study Results](#)

[Conclusions](#)

[Follow-Up Studies](#)

[Research with Other Salamander Subspecies](#)

[Hybridization Conclusions](#)

[Literature Cited](#)

Introduction

Introduced organisms can directly alter the genetic characteristics of native populations through *hybridization* and *introgression*. Here hybridization refers to breeding between individuals from two genetically different populations regardless of taxonomic rank (e.g. Rhymer and Simberloff 1996; Allendorf et al. 2001). Thus, hybridization can involve individuals from different *species*, different *subspecies*, or different populations of the same species or subspecies.

Introgression occurs when *first generation hybrids (F_1)* are fertile and backcross with the parentals also producing fertile progeny. After a few successive generations of backcrossing, introgression may result in the formation of *hybrid swarms*. That is, populations in which essentially all individuals are of hybrid origin. From a genetics perspective, the formation of a hybrid swarm represents *genomic extinction* because the genetic material from the native population is irretrievably mixed in evolutionarily novel combinations with that of the introduced organisms.

Tiger Salamanders

Tiger salamanders of the genus *Ambystoma* represent a good example of some of the conservation issues that hybridization and introgression pose. Tiger salamanders are a complex of about 15 species native to North America (Shaffer et al. 1991; Shaffer and McKnight 1996; Shaffer et al. 2004; Figure 1).

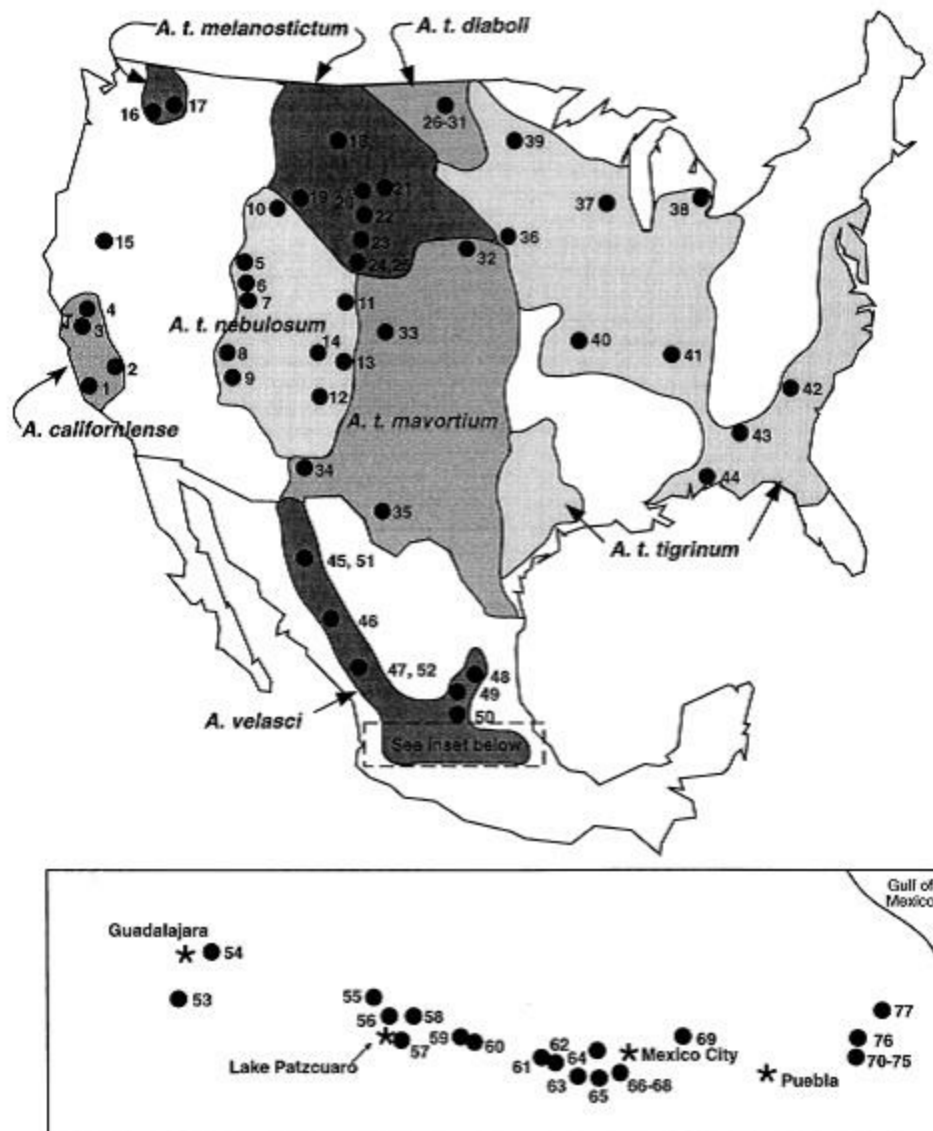


Figure 1. Geographical distribution of the tiger salamander species complex. Note that only two species are native to the United States the California tiger salamander *Ambystoma californiense* and at least five subspecies of *A. tigrinum*. Dots represent sample locations of Shaffer and McKnight (1996). From Shaffer and McKnight (1996).

In the United States, only two species are recognized. *A. tigrinum* is a polytypic species with five to seven recognized subspecies (e.g. Shaffer and McKnight 1996)

that is distributed throughout much of the continental United States and southern Canada (Figure 1). The California tiger salamander *A. californiense* is restricted to California's Central Valley and inner coast range (Figure 1). These two species had been completely reproductively isolated from each other for about five million years because of the Sierra Nevada uplift and subsequent formation of the Great Basin Desert. Thus, genetically they are highly divergent from each other (e.g. Shaffer and McKnight 1996; Figure 2).

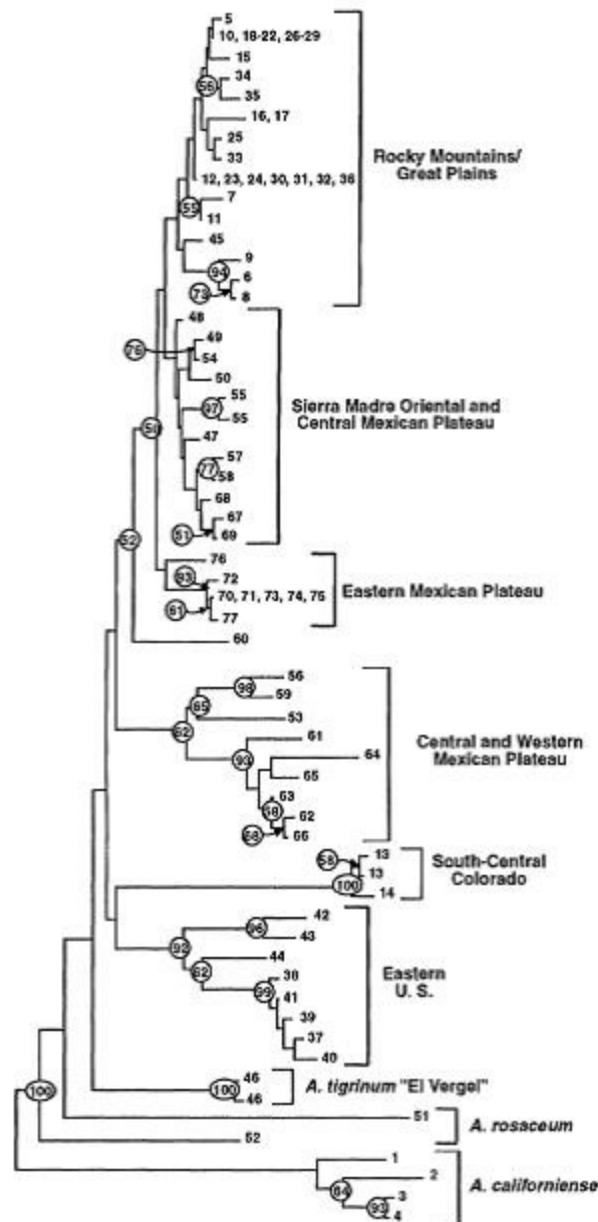


Figure 2. Proposed evolutionary or phylogenetic relationships of nine major lineages of tiger salamanders based on sequence data from 840 base pairs of mitochondrial DNA. From Shaffer and McKnight (1996). Note the California tiger salamander *A. californiense* appears to be the most genetically divergent lineage. The Eastern U. S. lineage is composed of *A. tigrinum tigrinum*. The South-Central Colorado lineage is composed of *A. t. nebulosum*. The Rocky Mountains/Great Plains lineage contains individuals classified as *A. t. nebulosum*, *A. t. melanosticum*, *A. t. diaboli*, *A. t. mavortium*, and *A. t. tigrinum*. Sample numbers refer to those in Figure 1. Numbers in circles represent

the probability that the observed branches are correct.

California Tiger Salamanders

California tiger salamanders exist in a Mediterranean climate characterized by rainy cool winters and hot dry summers (Riley et al. 2003). Historically they bred in vernal ponds that filled during the winter from November through March (Riley et al. 2003). The resulting larvae are obligate metamorphs meaning that in order to attain sexual maturity that they must metamorphose into the terrestrial form before the ponds dry by April through June (Petranka 1998; Riley et al. 2003). Upon metamorphosis, the juveniles migrate into upland grassland and open oak forest habitat usually less than 1km from the breeding site where they generally reside in mammalian burrows. Usually juveniles attain sexual maturity at the age of two or three but, at times not until age five. Adults can live ten years or slightly longer. When winter rains commence, adults migrate to breeding ponds but most individuals do not breed on an annual basis. Because of human modification of the environment, California tiger salamanders now breed in three different habitats: remaining vernal ponds, modified vernal or excavated ephemeral ponds for cattle watering that usually contain water longer than the vernal ponds, and perennial excavated landfill or gravel ponds (Riley et al. 2003; Fitzpatrick and Shaffer 2004).



California Tiger Salamander, photo by Pete Trenham, USFWS

The Research

Shaffer et al. (2004) analyzed a 734 *base pair (bp)* sequence of the *mitochondrial DNA (mtDNA)* control region of California tiger salamanders collected from throughout their range. The results indicated that based on this portion of the *genome* the salamanders are divisible into six genetically very divergent groups: highly isolated and divergent populations in the northern (Sonoma County) and southern (Santa Barbara County) edges of the species range and populations in the southern San Joaquin Valley, Central Coast Range, Central Valley, and the San Francisco Bay Area (Figure 3).

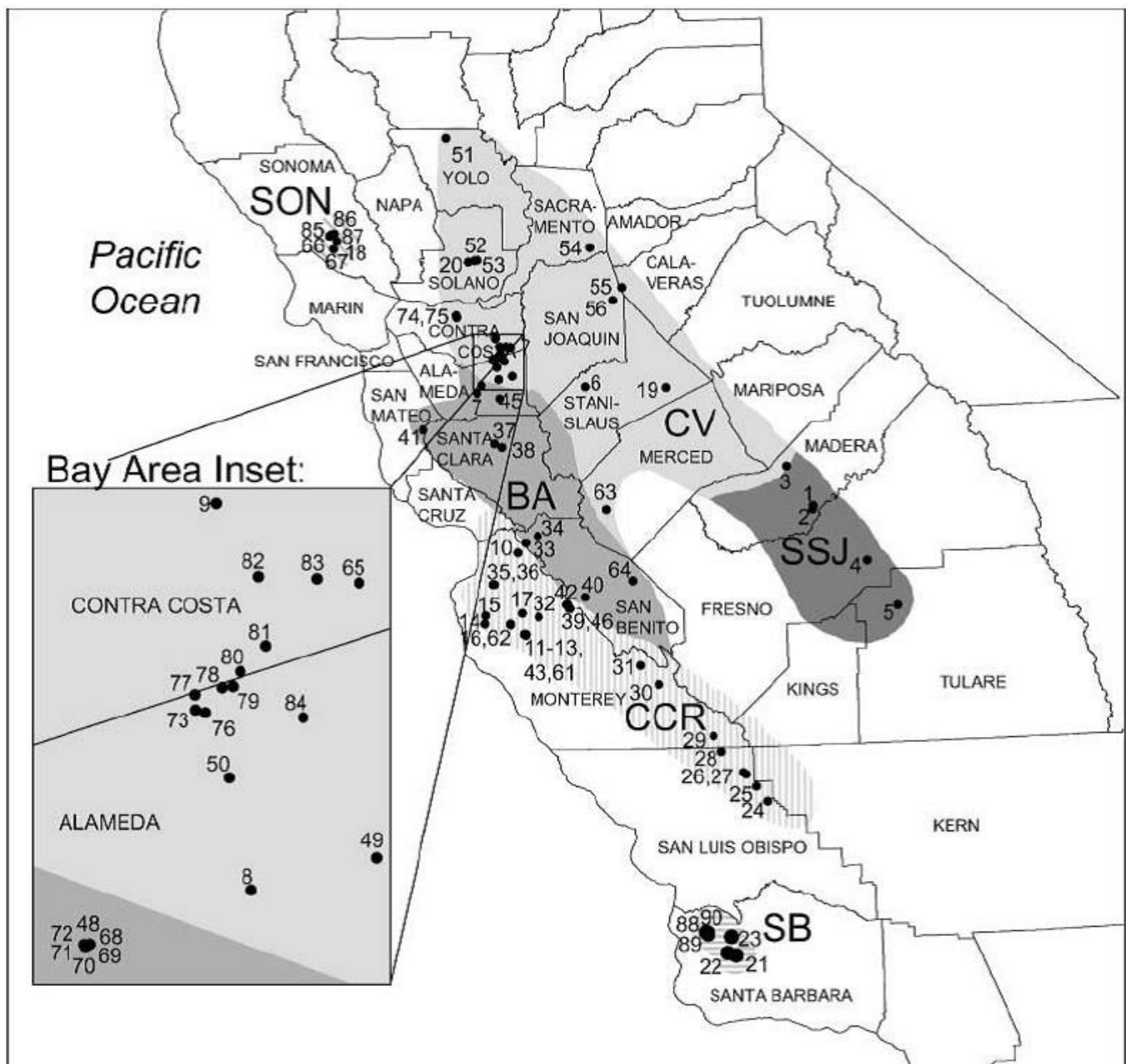


Figure 3. Geographic range of the California tiger salamander. BA=Bay Area group, CCR=Central Coast Range group, CV=Central Valley group, SB=Santa Barbara group, SON=Sonoma group, and SSJ=Southern San Joaquin group genetically identified by Shaffer et al. (2004). Dots represent sample locations of Shaffer et al. (2004). From Shaffer et al. (2004).

The latter four groups are separated by geological features, ecological zone boundaries, or both that constitute dispersal barriers. Among the six groups, 75% of the *haplotype* variability was due to differences among them, 18% to differences among breeding sites within groups, and only seven percent to variation within breeding sites. The latter value is probably somewhat underestimated as only two to 12 individuals were collected per site. Regardless of this, the results clearly suggest that historically there has been very restricted gene flow, if any, among the groups and that they essentially have been independent *evolutionary lineages* for a substantial period of time. Thus, Shaffer et al. (2004) suggest these groups as being strong candidates for recognition as *distinct population segments (DPS)* (USFWS and NMFS 1996). They, however, did not recommend taxonomic revision.

The Listing

Because of decreasing abundance, the United States Fish and Wildlife Service (USFWS) listed California tiger salamanders as threatened under the U. S. Endangered Species Act (ESA) in 2004 (USFWS 2004).



Although the USFWS recognized the Sonoma, Santa Barbara, and Central Valley which included the San Joaquin, Central Coast Range, Central Valley, and Bay Area groups identified by Shaffer et al. (2004) as DPS's, they considered all to be threatened and, therefore, instead of listing them separately they listed the entire species. The primary reason for decline was considered to be habitat destruction, degradation, and fragmentation due to conversion of rangeland into crop production and urbanization. Other reasons for decline were considered to be predation from introduced organisms such as bullfrogs *Rana catesbeiana* and mosquito fish *Gambusia affinis* and hybridization and introgression with the introduced barred tiger salamander *A. t. mavortium*.

Barred Tiger Salamanders



The barred tiger salamander is native to the south central United States (Figure 1). It was purposefully introduced during the 1940's and 1950's into the range of the California tiger salamander as a source of bait for fishermen targeting the introduced largemouth bass *Micropterus salmoides* (Riley et al. 2003; Fitzpatrick and Shaffer 2004, 2007a). Apparently almost all of the purposeful introductions occurred in the Salinas Valley in Monterey County which exists in the Central Valley DPS and the Central Valley group identified by Shaffer et al. (2004). Barred tiger salamanders were preferred for bait over California tiger salamanders because of their larger size, extended larval period, and in perennial ponds their ability to produce paedomorphic individuals (adults that retain the aquatic larval phenotype; Riley et al. 2003). The latter two aspects assure a more reliable source of bait for bait dealers and fisherman.

Investigating Hybridization

Riley et al. (2003) were the first to investigate hybridization between California and barred tiger salamanders. They collected larvae from two vernal ponds, two ephemeral cattle ponds, and two perennial landfill ponds in a one km² area in the Salinas Valley. In both the perennial ponds terrestrial adults were also collected and in one of these they also collected paedomorphs. This locale was chosen because it was known that barred tiger salamanders had been introduced to the area. Sample sizes per pond ranged from 33 to 61 individuals.

Restriction enzymes were used to assign mtDNA and *alleles* at two *nuclear genes* as having originated from California or barred tiger salamanders. For each individual a *hybrid index* was calculated as follows. California tiger salamander mtDNA and nuclear alleles were given a value of zero and barred tiger salamander mtDNA and nuclear alleles a value of one. These values summed over the mtDNA and nuclear genes yielded an individual's hybrid index. Thus, non-hybridized California tiger salamanders would have a hybrid index of zero and non-hybridized barred tiger salamanders a value of five. Since *F₁'s* must be *heterozygous* at both nuclear genes they would have a hybrid index of two or three depending on their mtDNA *haplotype*. Considering both mtDNA and nuclear genes, later generation hybrids (post F₁) could have one of 18 different combinations of California and barred tiger salamander mtDNA and nuclear alleles. Thus, their hybrid index could range from zero to five. Whether or not the samples appeared to have come from *hybrid swarms*, was tested by comparing the observed distribution of hybrid indices to the expected random distribution based on the proportion of California and barred tiger salamander mtDNA haplotypes and nuclear alleles detected. Statistical conformity to the expected random distribution would suggest the presence of a hybrid swarm.

Study Results

Individuals definitely of post F_1 hybrid origin and potentially non-hybridized barred tiger salamanders were detected in all the samples (Figure 4). Individuals potentially representing non-hybridized California tiger salamanders were detected in both vernal ponds and one such individual in one of the cattle ponds (Figure 4).

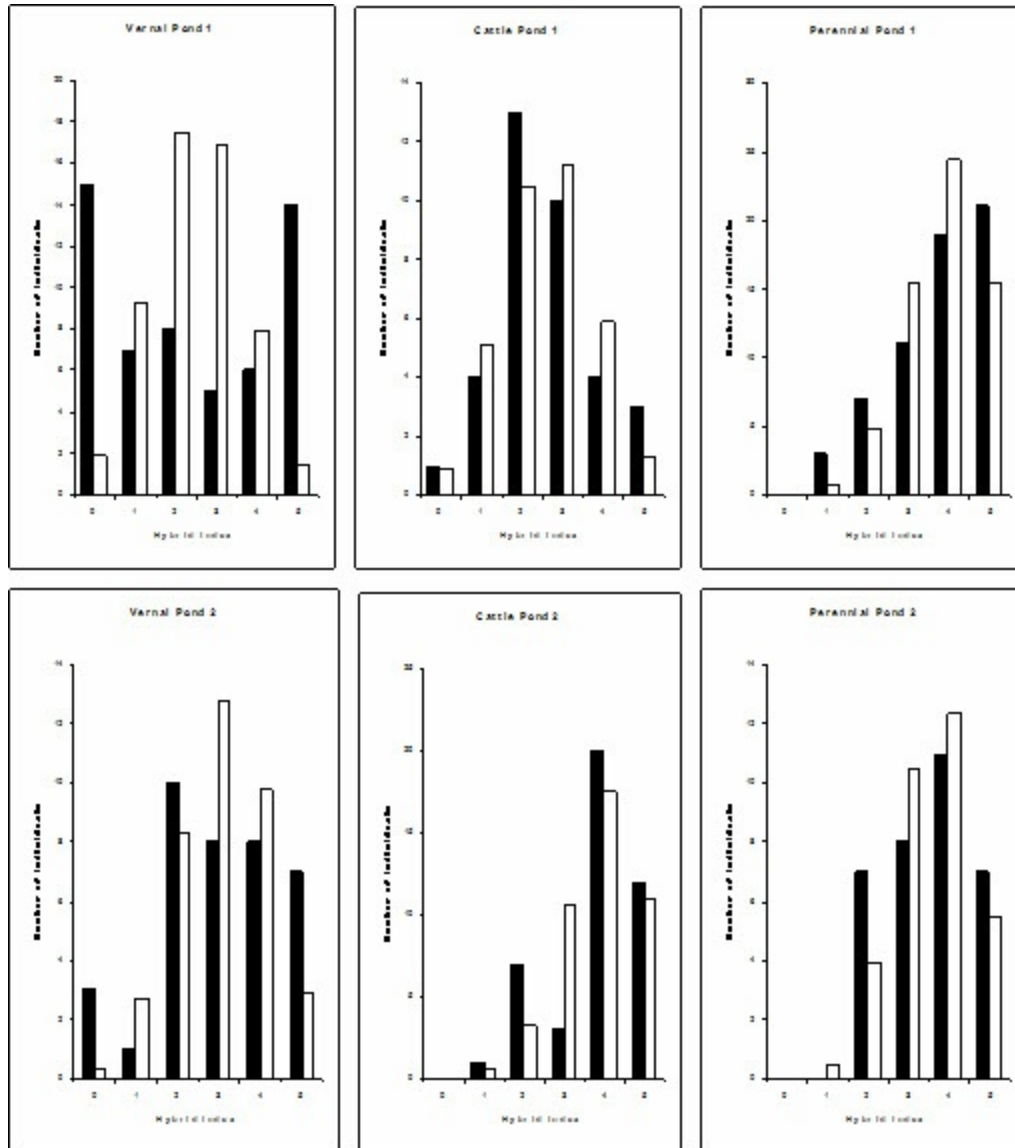


Figure 4. Observed (black bars) and expected random distribution (open bars) of hybrid index scores in samples from two vernal ponds, two ephemeral cattle ponds, and two perennial landfill ponds showing evidence of hybridization between native California and introduced barred tiger salamanders. In both the vernal pond samples, the observed distribution significantly differs from the expected random distribution indicating these samples did not come from hybrid swarms. In contrast, the observed distribution of hybrid indices in both the perennial and cattle ponds statistically conforms to the expected random distribution suggesting these samples came from hybrid swarms. This potential trend between mean proportion of California and barred tiger salamander mtDNA and nuclear alleles was further investigated by collecting larvae from four vernal, four cattle, and four perennial ponds in the Salinas Valley (Fitzpatrick and Shaffer 2004). Restriction enzymes were used to assign mtDNA and alleles at seven nuclear loci from each individual as being derived from California or barred tiger salamanders. As in Riley et al. (2003), from these data a hybrid index for each individual was calculated and in

each pond the observed distribution of hybrid indices was compared to the expected random distribution based on the average proportion of California and barred tiger salamander mtDNA and nuclear alleles detected. In this case, non-hybridized California tiger salamanders would have a hybrid index of zero, non-hybridized barred tiger salamanders a value of 15, F₁'s a value of seven or eight depending on their mtDNA, and post F₁'s could have any value from zero to 15.

In both the cattle and perennial ponds, the observed distribution of hybrid indices statistically conformed to the expected random distribution. Thus, these four ponds appeared to contain hybrid swarms suggesting that individuals with hybrid indices of zero or five in them actually were hybrids. In the vernal ponds, however, the observed distribution of hybrid indices significantly deviated from the expected random distribution due to an excess of individuals with zero and five values and a deficit of individuals definitely of hybrid origin. This suggests that these ponds probably contained some non-hybridized California and barred tiger salamanders. The deficit of salamanders definitely of hybrid origin suggests that either there is some *assortative mating* between the parentals in these ponds, selection against the hybrids, or that barred tiger salamanders only recently colonized the ponds and there has not been sufficient time for the formation of a hybrid swarm. The latter, however, seems unlikely given the close geographic proximity of the ponds and the fact that barred tiger salamanders are known to have been introduced to the area about 60 years ago.

Circumstantial evidence for selection is provided by the observation that the average proportion of California and barred tiger salamander mtDNA haplotypes and nuclear alleles is statistically heterogeneous (contingency table chi-square; $P < 0.001$) among the samples and appears to be somewhat associated with habitat type (Table 1).

Table 1		
Average frequency of California and barred tiger salamander mtDNA haplotypes and nuclear alleles in two vernal ponds, two excavated cattle ponds, and two excavated perennial landfill ponds containing hybrids between these species (Riley et al. 2003).		
Sample	Average frequency California	Average frequency barred
Vernal 1	0.52	0.48
Vernal 2	0.39	0.61
Cattle 1	0.48	0.52
Cattle 2	0.24	0.76
Perennial 1	0.24	0.76
Perennial 2	0.29	0.71

The average proportion of California tiger salamander mtDNA and nuclear alleles tends to be higher in the natural vernal ponds and lowest in the excavated perennial ponds. This apparent trend suggests that as the habitat becomes less "natural" that there may be less impediment to introgression from barred tiger salamanders.

On the average, samples from perennial ponds tended to have a higher proportion of barred tiger salamander mtDNA and nuclear alleles than the samples from vernal or cattle ponds (Figure 5). Furthermore, the observed distribution of hybrid indices statistically conformed to the expected random distribution in all four samples from the perennial ponds but, in only one of the cattle ponds and none of the vernal ponds (Figure 5). In general, therefore, perennial ponds appeared to contain hybrid swarms but, vernal and cattle ponds did not. Finally, as previously observed potentially non-hybridized California tiger salamanders were detected mainly in vernal ponds (Figure 5). Thus, overall there appeared to be little or no impediment to introgression from barred tiger salamanders in the perennial ponds and generally some resistance to the formation of hybrid swarms in vernal and cattle ponds.

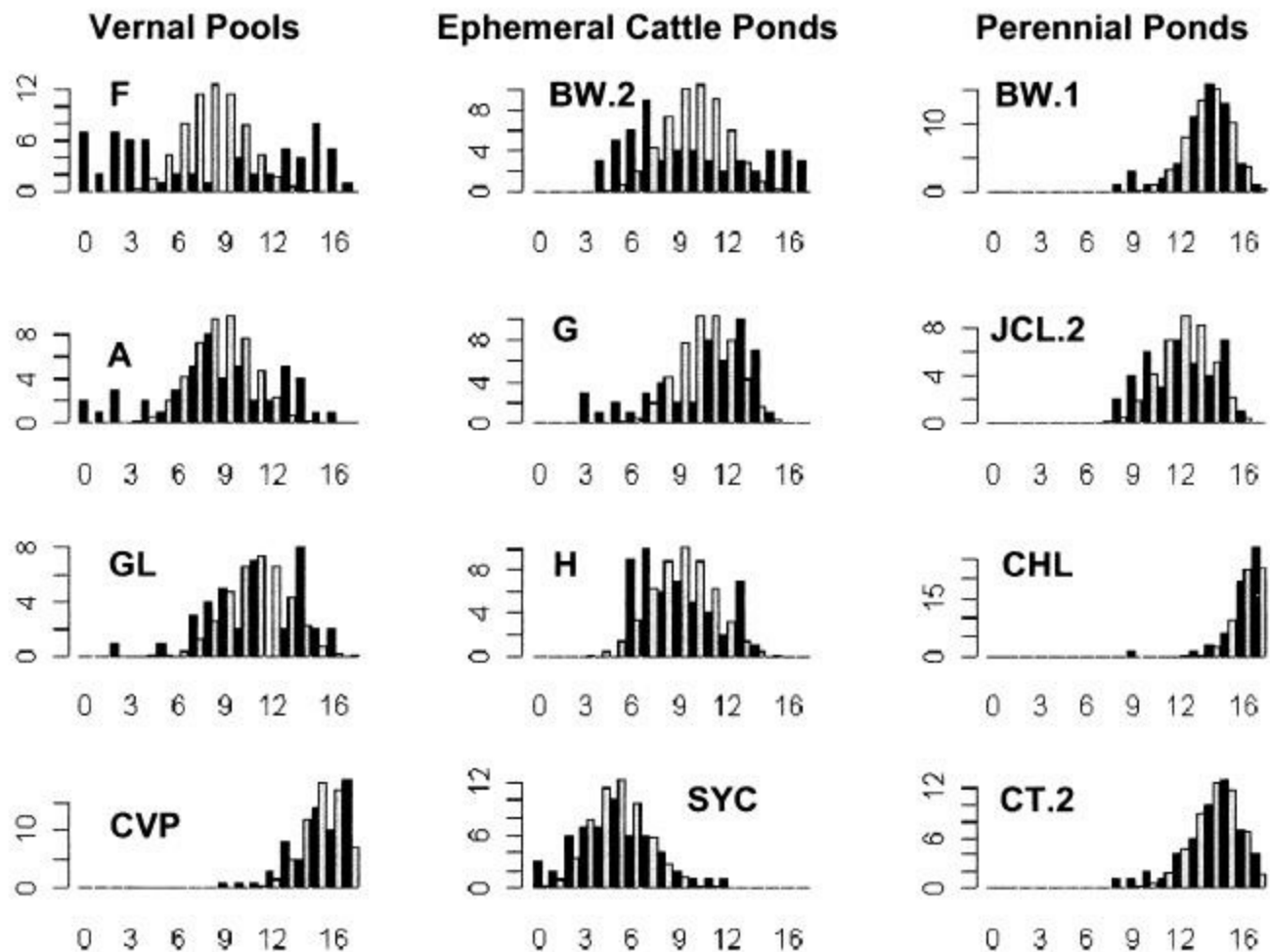


Figure 5. Observed (black bars) and expected random distribution (gray bars) of hybrid indices in samples from four vernal ponds, four ephemeral cattle ponds, and four perennial landfill ponds showing evidence of hybridization between barred and California tiger salamanders. Note the observed distribution statistically conforms to the expected random distribution only in the four perennial ponds and one of the cattle ponds (SYC). Thus, perennial ponds generally appear to contain hybrid swarms but, ephemeral vernal and cattle ponds do not. From Fitzpatrick and Shaffer (2004).

Conclusions

Fitzpatrick and Shaffer (2004) suggested that the above pattern of hybridization was a reflection of different *selection pressures* associated with habitat type among the ponds. They speculated that in perennial ponds barred tiger salamander alleles actually may be selected for because of being associated with increased reproductive success due to temporally extended breeding and paedomorphosis. In a non-hybridized barred tiger salamander population in a perennial pond in California outside the native range of California tiger salamanders, they noted that the barred tiger salamanders had a more extended breeding season than usually observed in non-hybridized California tiger salamander populations because of a tendency to begin breeding earlier in the fall. In hybrid populations, they postulated that earlier breeding associated with barred tiger salamander alleles would convey a competitive advantage to the larvae compared to those produced from later breeding individuals because of the increased size of the former.

Paedomorphs have been observed only in perennial ponds and all that have been analyzed contained barred tiger salamander alleles. Fitzpatrick and Shaffer (2004) speculated paedomorphs may have a reproductive advantage over terrestrial adults for two reasons. First, paedomorphic females are larger than terrestrial adults and, therefore, produce more eggs. Furthermore, paedomorphic females tend to breed earlier than terrestrial females again potentially conveying a competitive size advantage to their larvae.

Because of dispersal among ponds in the range of 20-30% (Trenham et al. 2001) and the close proximity of the ephemeral vernal and cattle ponds to perennial ponds, without some opposing selection the frequency of barred tiger salamander haplotypes and nuclear alleles should be much higher than is usually observed in the ephemeral ponds. With this amount of dispersal, after about ten generations the frequency of barred tiger salamander haplotypes and nuclear alleles should approach homogeneity among the ponds (Fitzpatrick and Shaffer 2004). The observed frequency of barred tiger salamander haplotypes and nuclear alleles in the ephemeral ponds, however, is highly heterogeneous and barred tiger salamanders have existed in the range of the California tiger salamander for well over ten generations. Thus, Fitzpatrick and Shaffer (2004) hypothesize there must be some selection against barred tiger salamander alleles in ephemeral ponds. The continued presence of hybrid individuals in these ponds is attributed to continual gene flow from perennial ponds. The genetic characteristics of the salamanders in these ponds, therefore, are considered to generally be reflective of selection opposing gene flow from perennial ponds.

Direct support for the non-neutrality of hybridization between California and barred tiger salamanders was provided by Fitzpatrick and Shaffer (2007b). They collected random samples of eggs from two vernal, one cattle, and two perennial ponds that previous studies indicated contained hybrids. They used the same *diagnostic markers* as their previous study (Fitzpatrick and Shaffer 2004) and compared the genetic characteristics of the eggs to a random sample of larvae collected about one to one and a half months post hatch. In all the ponds, they found that compared to the eggs the larvae had higher *heterozygosity* and more intermediate amounts of admixture (that is, a more equal proportion of California and barred tiger salamander alleles). This strongly suggests that larvae with more even admixed ancestry had increased survival during this early stage of life characterized by high mortality regardless of habitat type.

The above results, although compelling, cannot completely explain the trends observed by Shaffer and Fitzpatrick (2004). With selection for larvae with relatively even admixed ancestry, it would be difficult for the hybrid swarms in perennial ponds to have such a high frequency of barred tiger salamander alleles unless there was substantial gene flow from non-hybridized barred tiger salamanders. This seems unlikely because the gene flow would prevent the formation of hybrid swarms and few, if any, non-hybridized barred tiger salamanders appear to exist in the study area (Fitzpatrick and Shaffer 2004, 2007a). Although the observed selection for increased early survival is compatible with the more even frequency of California and barred tiger salamander alleles observed in the vernal and cattle ponds, without some subsequent opposing selection it cannot account for the general failure to observe hybrid swarms in these ponds. Thus, it appears that within ponds selection pressures vary temporally and possibly between larval and terrestrial life stages as in all ponds almost all individuals collected were larvae (Riley et al. 2003; Fitzpatrick and Shaffer 2004, 2007a, b). Not surprisingly, the genetic characteristics of the populations in these ponds appear to be influenced by the interaction of numerous factors both in space and time.

Follow-Up Studies

The previous studies all investigated hybridization by sampling ponds in the Salinas Valley in an area where it was known that barred tiger salamanders had been introduced. Thus, the prevalence of hybridization between California and barred tiger salamanders throughout the range of California tiger salamander was unknown. Fitzpatrick and Shaffer (2007a), therefore, collected larvae from 85 ponds spread throughout the Central Valley DPS. In 28 of these ponds, they looked for evidence of hybridization using the eight diagnostic markers used by Fitzpatrick and Shaffer (2004) and in the remaining 57 ponds they used the three diagnostic markers used by Riley et al. (2003).

There are a few notable aspects of their data. First, ponds containing a high frequency of barred tiger salamander alleles generally existed within 12km of known or strongly suspected introduction sites. Thus, almost all ponds within the Salinas Valley, where most introductions occurred, contained hybrids (Figure 6). The one exception was a highly isolated vernal pond complex in the northern part of the valley that appeared to contain non-hybridized California tiger salamanders (Figure 6). Outside the Salinas Valley, mainly non-hybridized California tiger salamanders were detected. Exceptions included two sites in the San Benito Valley, one of which was a known introduction site of barred tiger salamanders and the other was a site at which hybrids had been introduced. Other exceptions included a low level of admixture detected in three ponds in the Bay Area and two ponds in the San Joaquin Valley (Figure 6). The Bay Area ponds could represent natural gene flow but, this is highly unlikely for the San Joaquin ponds as they are separated by about 100km of unsuitable habitat from the nearest known introduction site. Thus, the presence of hybrids at these sites more likely represents an unknown purposeful introduction or an accidental introduction due to the use of salamanders as bait.

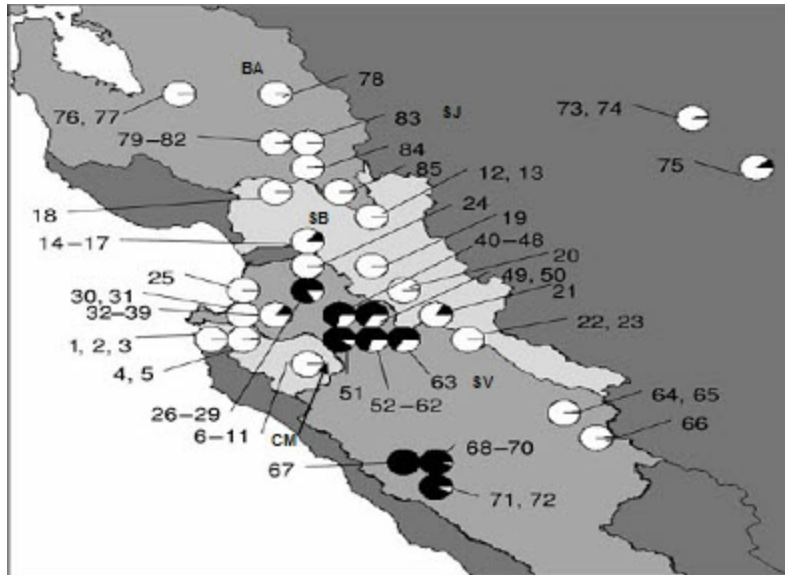


Figure 6. Locations of 85 ponds sampled by Fitzpatrick and Shaffer (2007a) within the Central Valley California tiger salamander DPS. Black areas of samples represent the average proportion of barred tiger salamander alleles detected. BA=Bay Area, CM=Carmel Valley, SB=San Benito Valley, SJ=San Joaquin Valley, and SV=Salinas Valley. From Fitzpatrick and Shaffer (2007a).

Overall, these data suggest that the introgression of barred tiger salamander alleles into California tiger salamanders has been a relatively slow process. After 50-60 years, it is still centered around introduction sites and the frequency of barred tiger salamander alleles generally drops to zero more than 12km from an introduction site. Thus, most populations showing evidence of hybridization exist within the Salinas Valley. At a large geographic scale, therefore, the extent of hybridization is mainly explained by introductions and low levels of long range gene flow. Within the Salinas Valley, the extent of hybridization is strongly associated with habitat type. At a smaller geographic scale, therefore, the extent of hybridization and introgression appears to mainly be a function of the interaction between selection and gene flow.

Research with Other Salamander Subspecies

Hybridization and introgression with introduced barred tiger salamanders is also a conservation issue for the Sonora tiger salamander *A. t. stebbinsi*. These animals have a very restricted natural distribution being found only in the San Rafael Valley of southeastern Arizona (Jones et al. 1988, 1995; Storfer et al. 2004). They are listed as endangered under the ESA (Gerst 1997). Historically they bred in perennial marshes and springs (cienegas) but, now because of human alteration of the habitat they almost exclusively breed in excavated, perennial cattle ponds (Storfer et al. 2004). Larvae may metamorphose into the terrestrial form but, most become paedomorphs (Storfer et al. 2004).

Storfer et al. (2004) sampled 26 cattle ponds located throughout the San Rafael Valley. They also obtained samples from 21 locations throughout the native range of the geographically proximate but allopatric Arizona tiger salamander *A. t. nebulosum*. Samples of barred tiger salamanders were obtained from 12 sites in the very southwestern portion of its native range in Arizona. The ranges of the Sonora and barred tiger salamanders in this area are adjacent to each other but, historically the two subspecies were allopatric.



They sequenced 916 bp of mtDNA from a total of 112 individuals from the 26 putative Sonora tiger salamander sites, ten barred tiger salamanders from six of the sample locations, and 24 Arizona tiger salamanders from 15 of the sample sites. Among these individuals only four haplotypes were detected. Haplotypes C and D were detected only and exclusively in the range of the Arizona tiger salamander suggesting these to be characteristic of this subspecies. Within the native range of the barred tiger salamander, only haplotype A was detected. Within the native range of the Sonora tiger salamander, both haplotypes B and A were detected but, 20 of the 26 sample locations only contained haplotype B. The presence of haplotype A in the range of both the Sonora and barred tiger salamander could be due to sharing of an ancestral polymorphism or the introduction of barred tiger salamanders into the range of Sonora tiger salamanders either with or without hybridization.

In order to attempt to distinguish between these possibilities, Storfer et al. (2004) examined the geographic range of haplotype A within the range of the Sonora tiger

salamander. They proposed that a random distribution would support the shared ancestral polymorphism interpretation. They hypothesized that ponds adjacent to roads would be the most likely sites for the introduction of barred tiger salamanders as a source of fish bait. Thus, a tendency for haplotype A to be clustered into locations adjacent to roads would favor the introduction interpretation.

Hybridization Conclusions

Data from all individuals from which mtDNA sequences were obtained as well as some additional ones were collected from nine microsatellite loci to address the hybridization issue. Excluding the six locations containing both haplotypes A and B, they combined all their data into a single set and used an iterative random assignment procedure to search for genetically distinct groups. After four iterations, all their specimens were divisible into three genetically distinct groups. These groups based upon the mtDNA haplotypes of individuals within them corresponded with one hundred percent accuracy to Arizona (C and D), barred (A), and Sonora tiger salamanders (B).

Based on the above data, there were no known diagnostic microsatellite loci among the subspecies. Thus, in order to investigate potential hybridization between Sonora and barred tiger salamanders they calculated a hybrid index for each individual in the barred and Sonora tiger salamander groups using the procedure of Campton and Utter (1985). In this analysis, Sonora tiger salamanders had values near one and barred tiger salamanders values around zero. Using the above data as a baseline, a hybrid index was calculated for each individual collected from the six sites in the range of the Sonora tiger salamander that potentially may have contained hybrids because they contained both haplotypes A and B. Finding some or all individuals from these sites with intermediate hybrid indices would be indicative of hybridization.

The A haplotype was significantly associated with ponds close to roads suggesting it was more likely introduced rather than an ancestral polymorphism. Furthermore, about 45% of the individuals from ponds having haplotypes A and B had intermediate hybrid indices, about 50% had indices falling within the range of the Sonora tiger salamander, and about five percent had values within the range of the barred tiger salamander (Figure 7). Taken together, therefore, these data strongly suggest hybridization has occurred between the introduced barred and native Sonora tiger salamander. The wide and continuous distribution of hybrid indices among the individuals from the ponds containing hybrids indicates that the hybridization has gone beyond the F_1 . The skewing of the hybrid indices of these individuals into the range of the Sonora tiger salamander indicates that the resulting introgression has mainly been from barred to Sonora tiger salamanders.

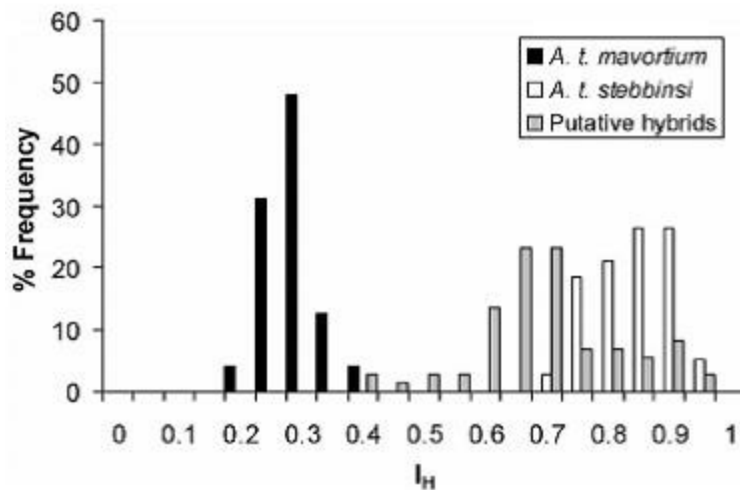


Figure 7. Distribution of hybrid indices (I_H) among non-hybridized barred tiger salamanders (*mavortium*), non-hybridized Sonora tiger salamanders (*stebbinsi*), and individuals from six ponds potentially containing hybrids between these two subspecies. From Storfer et al. (2004).

These examples of hybridization and introgression between native and introduced taxa raise the important but yet unresolved issue of what is the conservation value of hybrids and their legal status under the ESA. Historically hybrids were not accorded protection under the ESA. This policy, however, was questioned by some (e.g. O'Brien and Mayr 1991). Thus, the old policy was withdrawn and a new draft policy was proposed (USFWS 1996). This draft policy, however, was never officially adopted. Thus, one cannot look to the ESA for guidance on this issue as by default no official policy exists.

Allendorf et al. (2001) recognized six categories of hybridization; three of which they considered to represent natural hybridization and the others anthropogenic. They suggested that natural hybridization should not be considered a conservation issue but, that individuals and populations arising from anthropogenic hybridization generally have little or no conservation value. An exception to anthropogenic hybridization would be when hybrids constitute all or nearly all of the remaining populations. In this case, it was suggested hybrids would have conservation value as their continued persistence would essentially be the only option available to avoid the complete loss of the native genetic material.

Although not universally accepted (e.g. Campton and Kaeding 2005), the above guidelines suggest that since only a relatively small proportion of Sonora tiger salamander populations are hybridized that such populations should have little or no conservation value. Rather, efforts should be directed at protecting and conserving the remaining non-hybridized populations.

The situation of the California tiger salamander is not as straightforward. It appears that within the Salinas Valley essentially all populations are hybridized. The Salinas Valley constitutes almost the entire distribution of the Central Coast Range DPS proposed by Shaffer et al. (2004). Outside the Salinas Valley numerous non-hybridized populations of California tiger salamanders remain. Considering the species as a whole, therefore, numerous non-hybridized populations exist. Thus, at this level the hybridized populations would have little value. If one focuses only on the Central Coast Range DPS proposed by Shaffer et al. (2004), then few non-hybridized populations would exist and hybridized ones could be viewed as having conservation value. Adoption of the latter would suggest that different management and conservation strategies may be appropriate for the California tiger salamander. Within the Central Coast Range DPS hybrids would have conservation value. Outside this DPS they would have no value.

Literature Cited

- Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problem with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution* 16:613-622.
- Campton, D. E., and L. R. Kaeding. 2005. Westslope cutthroat trout, hybridization, and the U. S. Endangered Species Act. *Conservation Biology* 19:1323-1325.
- Campton, D. E., and F. M. Utter. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki clarki*) in two Puget Sound streams. *Canadian Journal of Fisheries and Aquatic Sciences* 42:110-119.
- Fitzpatrick, B. M., and H. B. Shaffer. 2004. Environment-dependent admixture dynamics in a tiger salamander hybrid zone. *Evolution* 58:1282-1293.
- Fitzpatrick, B. M., and H. B. Shaffer. 2007a. Introduction history and habitat variation explain the landscape genetics of hybrid tiger salamanders. *Ecological Applications* 17:598-608.
- Fitzpatrick, B. M., and H. B. Shaffer. 2007b. Hybrid vigor between native and introduced salamanders raises new challenges for conservation. *Proceedings National Academy of Sciences USA* 104:15793-15798.
- Gerst, J. L. 1997. Endangered and threatened wildlife and plants; determination of endangered status for three wetland species found in southern Arizona and northern Sonora, Mexico. *Federal Register* 62:665-689.
- Jones, T. R., J. P. Collins, T. D. Kocher, and J. B. Mitton. 1988. Systematic status and distribution of *Ambystoma tigrinum stebbinsi* Lowe (Amphibia: Caudata). *Copeia* 1988:621-635.
- Jones, T. R., E. J. Routman, D. J. Begun, and J. P. Collins. 1995. Ancestry of an isolated subspecies of salamander, *Ambystoma tigrinum stebbinsi* Lowe: the evolutionary significance of hybridization. *Molecular Phylogenetics and Evolution* 4:194-202.
- O'Brien, S. J., and E. Mayr. 2001. Bureaucratic mischief: recognizing endangered species and subspecies. *Science* 251:1187-1188.
- Petranka, J. W. 1998. *Salamanders of the United States and Canada*. Smithsonian

Institution Press, Washington D.C.

- Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. *Annual Reviews in Ecology and Systematics* 27:83-109.
- Riley, S. P., H. B. Shaffer, S. R. Voss, and B. M. Fitzpatrick. 2003. Hybridization between a rare, native tiger salamander (*Ambystoma californiense*) and its introduced congener. *Ecological Applications* 13:1263-1275.
- Shaffer, H. B., J. M. Clark, and F. Kraus. 1991. When molecules and morphology clash: a phylogenetic analysis of the North American Ambystomatid salamanders (Caudata: Ambystomatidae). *Systematic Zoology* 40:284-303.
- Shaffer, H. B., and M. L. McKnight. 1996. The polytypic species revisited: genetic differentiation and molecular phylogenetics of the tiger salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. *Evolution* 50:417-433.
- Shaffer, H. B., G. B. Pauly, J. C. Oliver, and P. C. Trenham. 2004. The molecular phylogenetics of endangerment: cryptic variation and historical phylogeography of the California tiger salamander, *Ambystoma californiense*. *Molecular Ecology* 13:3033-3049.
- Storfer, A., S. G. Mech, M. W. Reudink, R. E. Ziemba, J. Warren, and J. P. Collins. 2004. Evidence for introgression in the endangered Sonora tiger salamander, *Ambystoma tigrinum stebbinsi* (Lowe). *Copeia* 2004:783-796.
- Trenham, P. C., W. D. Koenig, and H. B. Shaffer. 2001. Spatially autocorrelated demography and interpond dispersal in the salamander *Ambystoma californiense*. *Ecology* 82:3519-3530.
- USFWS (United States Fish and Wildlife Service). 1996. Endangered and threatened wildlife and plants; proposed policy and proposed rule on the treatment of intercrosses and intercross progeny (the issue of hybridization); request for public comment. *Federal Register* 61:4710-4713.
- USFWS (United States Fish and Wildlife Service). 2004. Endangered and threatened wildlife and plants; determination of threatened status for the California tiger salamander; and special rule exemption for existing routine ranching activities; final rule. *Federal Register* 69:47212-47248.
- USFWS (United States Fish and Wildlife Service) and NMFS (National Marine

Fisheries Service). 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register 61:4722-4725.